



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

001459

OFFICE OF  
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: February 11, 1982

SUBJECT: Tebuthiuron technical, repeat rat re<sub>o</sub>production study,  
Accession No. 246374, TB/HED comments on.

FROM: Mary L. Quaife, Ph.D. *MLQ, 2/11/82*  
Toxicology Branch/HED (TS-769) *fig 2/15/82*

TO: PM, Mr. R. Taylor  
Registration Division (TS-767)

EPA Reg. No. 1471-109  
(TB) Caswell No. 366AA

E. Lilly and Company  
Indianapolis, Indiana 46285

CONCLUSIONS:

1. The study ( preliminary review of which is attached) is judged to conform adequately with submitted and approved (4/17/80) protocol for it.
2. However, in order to complete our review and inter<sub>o</sub>pretation of the study, TB/HED requests following information of E. Lilly and Company:
  - "A. Show: The model for ANOVA or ANCOVA with your rationale for the model; the expected mean square errors; the tables showing the computed mean square errors with associated degrees of freedom; and the means, the variance-covariance matrices, and other pertinent EDP outputs associated with the significance statements relating to absolute body weights of the parents and the weanlings of the  $F_1$  and  $F_2$  generations.
  - B.
    - i. Why is the Bonferroni adjustment applied to results of Dunnett's "t" test?
    - ii For which comparisons will the inference of statistical significance be materially affected if not used (i.e., which comparison related to body weight will become statistically significant at  $p \leq 0.05$ )?"

1/11

NEW TOXICOLOGY:

"A two-generation reproduction study with tebuthiuron (compound 75503) in the Wistar rat," by E. R. Adams, N. V. Cwen, and J. A. Hoyt, E. Lilly & Co., Greenfield, Ind. 46140, November, 1981, Acc. No. 246374; Lilly Nos. RC3780 and RO8780.

Test compound. Tebuthiuron (N-[5-(1,1-dimethylethyl)-1,3,4-thiadiazol-2-yl]-N,N'-dimethylurea) technical, Lot No. 00880-1L-1, X-35920, analytical characterization according to attached pages, Appendices C-1 to C-3, this report (copies of), p. 9-11 of this memo.

Procedure. Groups of 25M and 25F Wistar rats (Harlan Industries, Cumberland, Indiana) each received 0, 100, 200, or 400 ppm test compound in the diet (Purina mash) for 101 days (Fo rats) or 124 days (F1 rats) and then for a further period sufficient to mate and rear (ca. 9-10 weeks apart) two successive litters of young to 21 days of age. F1a rats were parents of the F2 offspring.

At the start of feeding tebuthiuron, Fo rat males weighed 100 g and females 90 g (mean each), and F1 rat males and females were ca. 5 wks old.

Prior to mating, all rats were weighed weekly. Males were weighed monthly, thereafter, and females were weighed on the day a copulatory plug was found; on gestation day 20; and on postpartum day 21. Progeny were weighed individually on postpartum days 1, 4, 7, 14, and 21. Rats were culled to 10 per litter on day 4 - with equal numbers of each sex being left, if possible - by a random procedure (described in the report).

During growth, rats were observed daily for general condition, and they were examined more closely each week. Food consumption was measured weekly when rats were weighed, and cumulative efficiency of food utilization was calculated. Bred F's were observed near parturition and pups, when weighed.

Diet assay having shown tebuthiuron to be stable that long, diets were prepared either weekly or every two weeks. Samples of each diet were analyzed for tebuthiuron content at beginning of growth of each parent generation and at termination of the study. Rats received diet and water (chlorinated city water) ad lib.

Sperm morphology and testes of 10 Fo males/dose-group were evaluated microscopically after the second breeding period. At the end of the study, ten F1 adults/sex/dose-group were examined grossly and following tissues taken for microscopic evaluation: Kidney, liver, heart, lung, spleen, thymus, lymph node, salivary gland, pancreas, stomach, duodenum, jejunum, ileum, colon, ovary, uterus, adrenal, thyroid, testis, prostate, skin, mammary gland, skeletal muscle, and urinary bladder. Five weanlings/sex/dose-group of both F1a and F2a progeny were similarly examined, grossly and microscopically.

Gestation length was determined, and numbers of live and stillborn in each litter and number and condition of surviving progeny on postpartum days 1, 4, 7, 14, and 21 were determined for all offspring. Sex of each pup surviving to day 21 was determined (as was that of both culled and remaining progeny on day 4).

Following values were calculated for each dose-group and generation: Fertility index - proportion of females that were pregnant; gestation-survival index - proportion of newborn pups that were alive; survival index - proportion of offspring that survived to 1, 4, 7, 14, and 21 days; and mean number of offspring raised to weaning (day 21)/pregnant female.

Statistical comparisons of mean body weight; body weight gain; food consumption; and efficiency of food utilization were each made. Maternal body weights; gestation survival; liveborn litter size; progeny survival; and mean progeny weight (per litter) on postpartum day 21 were similarly evaluated, i.e., by Dunnett's two-tailed "t" test, with use of a Bonferroni "t," where appropriate. Chi-square contingency tables were used to evaluate fertility data, and statistical significance was set at  $p \leq 0.05$ .

Report states, in selecting Fla weanlings as parents of F2 rats, representatives of all available litters were included. Nor were individuals of poor physical condition or which weighed less excluded. Weanling selection from within a litter was based on a random number system.

Also, in second breeding trials in Fo and F1 generations, adult females failing to deliver were killed and examined for evidence of pregnancy. All remaining rats - Fo and F1 adults after second breeding trials; progeny culled on postpartum day 4; and weanlings not selected to continue the study - were given terminal eye and physical examinations and killed with CO<sub>2</sub> gas.

A diagram of plan of this study is reproduced on p. 8 of this memo.

#### Results.

Diet Assays. Mean values (only) for each dose-level diet for the three intervals sampled show good agreement between theoretical and found content of tebuthiuron, the largest difference in one diet, 40 ppm (160 vs. 200) or -20%.

Actual tebuthiuron intake. Time-weighted average tebuthiuron intake for each group - in order, from controls to highest dose-level - was 0, 7, 14, or 28 mg/kg body weight/day. Values are for (Fo, F1) rat pre-mating phases.

Mortality of parents and gross findings. No Fo rats died. Four high-dose rats, one low-dose rat, and one control in the F1 generation died, - apparently of causes unrelated to test compound.

Physical signs. No physical signs shown were judged related to treatment.

Food consumption and cumulative efficiency of food utilization. Tables show beginning body weight, body weight gain, and efficiency of food utilization for Fo and F1 rats during pre-mating periods - mean values/sex/dose-level:

Ppm TBZ in diet	M body wt at start, g	M body wt gained, g	M mean E.F.U.	F body wt at start, g	F body wt gained, g	F mean E.F.U.
Values for Fo rats, 25/sex/dose-level - 98 days						
0	103.4	446.0	17.4	93.4	265.0	13.3
100	101.2	461.0	17.6	88.5	265.9	13.7
200	99.4	460.3	17.5	87.1	257.0	13.2
400	89.1	442.9	17.3	89.5	251.1	13.1
Values for F1 rats, 25/sex/dose-level* - 128 days						
0	151.4	490.2	14.0	133.7	269.8	10.3
100	152.0	472.3	13.3	125.3	272.3	10.4
200	144.2	484.7	13.8	121.4	250.4	9.9
400	144.5	472.8	13.1*	118.2	232.5**	9.0*

\* Different for 100-ppm males, i.e., 24/sex/dose-level for them.

\*\* Differs from control ( $p \leq 0.05$ )

There were no treatment-related variations in mean daily food consumption during the respective Fo and F1 pre-mating periods.

Mean cumulative efficiency of food utilization (E.F.U.) of Fo test rats of both sexes did not vary from control values during the 98 days it was measured. High-dose (400-ppm) F1 rats had lower ( $p \leq 0.05$ ) E.F.U. near the end of 124 days' (total) feeding (males) or from day 98 on (females); while E.F.U. of F1 females at 200 ppm showed borderline depression.

Body weight of adult rats. Significantly ( $p \leq 0.05$ ) depressed mean body weights during pre-mating period were shown by female F1 rats at both 200 and 400 ppm, days 14 through 124 (i.e., virtually through the entire period). Respective day-124 mean body weights, with standard deviation, are shown below, as are corresponding values for controls and 100-ppm F1 females:

PPM TBZ in Diet	Mean body weight of F1 females at 124 days (g)	Standard deviation
0	403.4	$\pm 59.5$
100	397.6	$\pm 39.9$
200	371.8*	$\pm 38.4$
400	350.7**	$\pm 33.3$

\*  $P \leq 0.05$ , Dunnett's two-tailed t test, significance of difference from control.

\*\*  $P \leq 0.01$ , Dunnett's two-tailed t test, " " " " "

[As can be seen from table on preceding page, mean body weight gain during this period was significantly depressed in female F1 rats at 400 ppm and moderately, but not significantly, depressed in those at 200 ppm.]

Although the report notes (correctly) that the depression in mean F1 female weights in the middle and high dose groups continued throughout the two breeding trials, we do not find particular significance in this fact; since E.F.U. data are lacking and since weight gain would be expected to vary with number of fetuses carried by the pregnant rat.

Statistically significant differences of test rat values from corresponding control values were not seen for mean body wts of treated Fo parental male and female rats and F1 parental male rats during pre-mating periods or ensuing breeding trials.

Live-born litter size. Table below shows mean live-born litter size (and S.E.):

Rat progeny	Mean number liveborn per litter and S.E.			
	ppm tebuthiuron in diet			
	0	100	200	400
Fla	11.9 (1.0)	13.3 (0.7)	12.9 (0.7)	12.5 (0.7)
Flb	10.2 (1.2)	12.3 (0.9)	10.6 (0.8)	12.4 (0.8)

(continued)

Rat progeny	Mean number liveborn per litter and S.E. ppm tebuthiuron in diet			
	0	100	200	400
F2a	10.5 (1.1)	11.0 (1.2)	9.6 (0.9)	13.3 (0.6)
F2b	9.9 (1.3)	11.8 (1.2)	11.6 (1.1)	12.7 (0.9)

was

In general, treated females had larger litters than control females, but differences are said not to be statistically significant.

Fertility. Fertility of control rats appears low, fertility indices for the four litters (Fla-F2b, inclusive) varying from 48 to 68%. No reason is given for this. However, in all cases, test litters had higher mean fertility indices (varying from 64 to 96% for the three dose-levels of Fla-F2b litters, inclusive) than corresponding controls.

Gestation length. Mean gestation lengths were similar in all test groups, varying between 21.9 and 22.5 days.

Progeny survival. Mean gestation survival of all rats - test and control - was 93%, limits being 88 and 98%. Survival to 21 days is said to have varied from 76 to 100%. There was no effect of test compound on survival of offspring.

Sex distribution of progeny. Sex distribution of survivors was determined only on day 21. It was not affected by treatment and varied between 45 and 61% males.

Mean number of offspring surviving to 21 days of age per pregnant female. Mean number of offspring at day 21 per pregnant female varied between 6.3 and 8.1 for control groups in the four (Fla-F2b, inclusive) litters. Corresponding test animal values varied between 6.1 and 8.6. Thus, test and control values cover similar ranges.

Condition of progeny. No findings in test rat offspring are ascribed to tebuthiuron, either gross or microscopic (latter in 5 rats/sex/dose-level of Fla and F2a weanlings which were examined histopathologically). One high-dose pup had cleft palate and three pups within a single litter in the low-dose group had multiple anomalies, including rudimentary tail, imperforate anus, varus hind limbs, missing ribs bilaterally, missing vertebrae, and fused vertebrae. Due to low incidence and lack of dose-response, findings are not considered treatment-related (this reviewer agrees).

Adult rat examination results. No tissue alterations found in the Fla adults examined histopathologically are ascribed to treatment with the test chemical. Testes from control and test rats, Fo parental rats, which were examined microscopically were normal. Sperm morphology, also, was normal in all treated rats examined, i.e., Fo rats.

Body weights of offspring. Below are given mean progeny weights at day 1 and at day 21 of all rat progeny (with S.E.).

Ppm TBZ in the diet	Number of pregnant females	Mean body weight (g) $\pm$ S.E. of progeny on postpartum day		Progeny of litter
		1	21	
0	17	6.9 (0.2)	45.3 (1.6)	Fla
100	21	6.5 (0.2)	42.7 (1.7)	"
200	18	6.4 (0.1)	44.2 (2.5)	"
400	23	6.8 (0.2)	41.9 (1.7)	"
0	14	6.9 (0.1)	45.6 (1.7)	Flb
100	20	6.5 (0.2)	43.1 (1.2)	"
200	17	6.5 (0.1)	43.4 (1.8)	"
400	24	6.6 (0.1)	42.0 (1.1)	"
0	12	6.9 (0.3)	46.1 (2.0)	F2a
100	16	6.5 (0.2)	41.1 (1.6)	"
200	21	6.4 (0.2)	44.5 (1.8)	"
400	17	6.3 (0.1)	43.6 (1.0)	"
0	14	6.8 (0.2)	45.9 (2.2)	F2b
100	19	6.4 (0.2)	42.7 (1.4)	"
200	19	6.8 (0.2)	43.5 (1.7)	"
400	20	6.6 (0.1)	42.3 (1.2)	"

Without exception, mean 21-day body weights of test rat offspring are lower than corresponding controls. However, significant differences do not occur. (These remarks pertain to the table of preceding page of memo.)

Petitioner has calculated the mean 21-day body weights of test and control rat progeny in which 10 pups/litter were maintained "through postpartum day 21" presumably, meaning from day 4 (when litters were culled to 10 if necessary) to day 21. "Pooled means from the four breeding trials using the litter as the sampling unit" are given as 41.9, 41.4, 41.5, and 41.9 g, respectively, for control, low-dose, mid-dose, and high-dose offspring of the combined Fl<sub>a</sub>, Fl<sub>b</sub>, F2<sub>a</sub>, and F2<sub>b</sub> litters.

[In sum, with exception of retarded growth reported by Petitioner for dietary levels, 200 and 400 ppm, in this study, adverse effects are not reported.]

COMMENT:

This study review is incomplete. We need to be sure that proper statistical evaluation of parental body weights (or growth) prior to mating and, also, of weanling litter weights (and growth) is made. See below.

We have consulted our TB/HED Statistician, Mr. Bert Litt, over some questions we have (related to the above) in interpreting study results. He suggests we ask Petitioner for more information about the statistical analysis made on the study, as follows:

"Please answer questions as to how data were statistically analyzed:

- A. Show: The model used for ANOVA or ANCOVA with your rationale for the model; the expected mean square errors; the tables showing the computed mean square errors with associated degrees of freedom; and the means, the variance-covariance matrices, and other pertinent EDP outputs associated with the significance statements relating to absolute body weights of the parents and the weanlings of the Fl and F2 generations.
- B. 1. Why is the Bonferroni adjustment applied to results of Dunnett's "t" test?  
2. For which comparisons will the inference of statistical significance be materially affected if not used (i.e., which comparison related to body weight will become statistically at  $p \leq 0.05$ )?"

TB/HED withholds further comment on the study, pending receipt of replies to these questions (and TB/HED - Statistician's - evaluation of them).

[We note that correct interpretation of this study is very important. Up to now, we have declined to estimate an allowable daily intake (ADI) for tebuthiuron tech. due to apparent growth inhibition at lowest dietary level previously tested, 400 ppm, - in rat chronic and subchronic feeding trials - and to statistically significantly retarded growth of weanlings (and parents?), at 400 ppm, in previously submitted rat reproduction study. (No long-term dog study is available, on which an ADI might be based.) Therefore, an ADI will rely, presumably, in whole or in part, on the growth "no-effect level" determined for this reproduction study.]

EPA REG. NO. 105501

RIN 0634-93

Page \_\_\_\_\_ is not included in this copy.

Pages 8 through 11 are not included.

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